Archives of Insect Biochemistry and Physiology 54:47–54(2003)

Molecular Cloning and Characterization of a cDNA Encoding Cytochrome c Oxidase Subunit Va From the Lesser Grain Borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

Jorge Ayala,[1] Alan K. Dowdy,[2] Richard W. Beeman,[3] and Kun Yan Zhu[1]*

A cDNA encoding subunit Va of cytochrome c oxidase (EC 1.9.3.1) was cloned and characterized from a lesser grain borer (*Rhyzopertha dominica*) cDNA library. The complete cDNA consists of 693-bp and contains an open reading frame of 450-bp that encodes 150 amino acid residues. The sequence includes a 28-bp putative N-terminal and a 122-bp putative mature protein. The estimated molecular weight and pI for the predicted mature protein are 13,962 and 4.60, respectively. The cDNA-deduced amino acid sequence of the mature protein shows 73% identity to that of a corresponding subunit of African malaria mosquito (*Anopheles gambiae*) and 59% identity to that of the fruit fly (*Drosophila melanogaster*). In addition, 31% of all amino acid residues are conserved among six different animal species. Evolutionary distance analysis suggests that cytochrome c oxidase subunit Va from *R. dominica* is most similar to the corresponding subunit from the malaria mosquito. Northern analysis revealed a single 4.9-kb transcript that is much larger than that found in mammalian species. Arch. Insect Biochem. Physiol. 54:47–54, 2003.

KEYWORDS: amino acid sequence; cytochrome c oxidase; lesser grain borer; molecular cloning; *Rhyzopertha dominica*.

INTRODUCTION

Cytochrome c oxidase (EC 1.9.3.1) is the terminal component of the mitochondrial respiratory chain and plays a vital role in oxidative phosphorylation in both prokaryotes and eukaryotes (Taanman and Williams, 2001). The enzyme catalyzes one-electron oxidation of four reduced cytochrome c molecules and four-electrons reduction of one O_2 molecule (Myers and Palmer, 1988). In addition, the enzyme activity contributes to maintaining a transmembrane proton gradient by reducing O_2 to water and by coupling the translocation of protons across the mitochondrial membrane (Hatefi, 1985).

Crystallization of cytochrome c oxidase has led to the determination of the complete molecular structure in *Bos taurus* (Tsukihara et al., 1996) and *Paracoccus denitrificans* (Iwata et al., 1995). Mammalian cytochrome c oxidase consists of 13 differ-

[1]Department of Entomology, Kansas State University, Manhattan

[2]Center for Plant Health Science & Technology, United States Department of Agriculture, Animal & Plant Health Inspection Service, Plant Protection & Quarantine, Raleigh, North Carolina

[3]Grain Marketing and Production Research Center, Agricultural Research Service, U.S. Department of Agriculture, Manhattan, Kansas

Contract grant sponsor: USDA/ARS.

*Correspondence to: Kun Yan Zhu, Department of Entomology, 123 Waters Hall, Kansas State University, Manhattan, KS 66506-4004, E-mail: kzhu@ksu.edu

Received 26 March 2003; Accepted 19 May 2003

DOI: 10.1002/arch.10101
Published online in Wiley InterScience (www.interscience.wiley.com)

ent polypeptide subunits (Tsukihara et al., 1996). The three largest subunits (I, II, and III) are encoded by mitochondrial genes. These catalytic subunits are involved in redox-linked proton pumping (Zhang and Capaldi, 1988). The other 10 smaller subunits (IV, Va, Vb, VIa, VIb, VIc, VIIa, VIIb, VIIc, and VIII) are encoded in the nucleus and synthesized in the cytoplasm (Capaldi, 1990). The number of these nuclear subunits varies in eukaryotes. The nuclear subunits carry an N-terminal signal peptide that targets them to the mitochondria (Colman and Robinson, 1986; Hurt and van Loon, 1986). It has been suggested that the nuclear subunits modulate the activity of cytochrome c oxidase by regulating respiration and proton translocation according to the metabolic requirements of different tissues (Kadenbach, 1986). This may be the reason for the presence of different isoforms found in different mammalian tissues (Kadenbach, 1986; Grossman and Lomax, 1997).

Subunits Va, Vb, and VIb are extramembrane-subunits (Tsukihara et al., 1996). Subunits Va and Vb are located in the matrix side (the innermost space) whereas subunit VIb is located on the cytosolic side of the mitochondrion. Subunit Va is the most conserved subunit among mammals (Kadenbach and Reimann, 1992), and it may impede ATP inhibition of respiration by binding thyroid hormones, such as diiodothyronines, that stimulate mitochondrial activity (Arnold et al., 1998; Soboll, 1993).

Cytochrome c oxidase is a target enzyme of phosphine, an insecticidal fumigant that has been widely used to control stored grain insects (Nakakita et al., 1971; Chefurka et al., 1976). Phosphine poisoning in animals is manifested by respiratory inhibition (Nakakita, 1987; Chaudhry, 1997). In some regions of the world, the lesser grain borer (*Rhyzopertha dominica*) has developed resistance to phosphine. Because subunit Va is the most conserved subunit of cytochrome c oxidase among different animal species and may play an important role in modulating the activity of cytochrome c oxidase, we decided to clone and characterize its full-length cDNA from lesser grain borer, a major cosmopolitan stored product pest. In this study, we report: (1) the cDNA sequence of cytochrome c oxidase subunit Va and its conceptual translation; (2) the primary structure and characteristics of this subunit; and (3) similarities of the subunit to orthologs found in other animal species. This study was intended to help us understand the molecular structure and the function of cytochrome c oxidase in insects.

MATERIALS AND METHODS

Insect Culture

A phosphine-susceptible colony of *R. dominica* was obtained from the Grain Marketing and Production Research Center, U.S. Department of Agriculture/Agricultural Research Service (USDA/ARS) in Manhattan, Kansas. The insects were reared on whole wheat at 30 ± 2°C and 65 ± 5% RH.

Total RNA and poly(A) RNA isolations

Total RNA was isolated from *R. dominica* adults by using Trizol reagent (Life Technologies, Gaithersburg, MD). Poly(A) RNA was purified from total RNA using the MessageMaker Reagent Assembly according to the manufacturer's instructions (Life Technologies).

Generation of DNA probes

A homologous DNA probe for cytochrome c oxidase subunit Va was generated by hemi-nested PCR using lesser grain borer cDNA template and degenerate primers, based on the strategy described by Zhu and Clark (1995). The first round of PCR was carried out with the forward primer 5′GAR WSN GMN GAR GAR TTY GA and the reverse primer 5′TTR TCN WRN CCN ARY TCY TC. The second round of PCR was carried with the nested forward primer 5′GTN CCN GAN CCN AAR ATH AT and the same reverse primer as that of the first round of PCR. The PCR program for both rounds consisted of a denaturation cycle at 95°C for 1 min; 35 cycles at 95°C for 1 min, 50°C for 1 min, and 72°C for 3 min for denaturation, annealing, and

extension, respectively; and a final extension cycle at 70°C for 10 min.

The PCR-amplified DNA fragment was subcloned into a pCR-Blunt vector using the Zero Blunt PCR cloning kit (Invitrogen, Carlsbad, CA). Five clones were isolated, and plasmid DNA was extracted using a QIAprep Miniprep kit (Qiagen, Valencia, CA). Vector inserts were verified by PCR and agarose gel electrophoresis. Nucleotide sequence was determined using an ABI PRISM 3700 DNA analyzer (Foster City, CA) at the Sequencing and Genotyping Facility, Kansas State University, Manhattan, KS.

The DNA fragment corresponding to cytochrome c oxidase subunit Va was isolated from the plasmid vector by restriction digestion, agarose gel electrophoresis, and DNA extraction using the Wizard PCR Preps DNA purification system (Promega, Madison, WI). The fragment was then labeled with α-[^{32}P]dATP (PerkinElmer, Life Sciences, Boston, MA) using a nick translation labeling system (Promega). The radioactive probe was used for both cDNA library screening and Northern blot analysis.

Construction of cDNA Library

An *R. dominica* cDNA library was constructed using the ZAP-cDNA synthesis kit and the ZAP-cDNA Gigapack III gold cloning kit according to the manufacturer's instructions (Stratagene, La Jolla, CA). Double-stranded cDNA was synthesized from 5 µg of poly(A) RNA, blunt-ended with 5 U of *Pfu* DNA polymerase, and then ligated to the *Eco*R I adapters. The ends of the adapters were then phosphorylated and digested with *Xho* I. The cDNA was then resolved on a cDNA size fractionation column (Life Technologies), and 100 ng of the product was ligated into the Lambda Uni-ZAP XR vector. The ligated DNA was packaged with the Gigapack III Gold packaging extract.

cDNA Library Screening

Recombinant clones were grown on agar plates of the bacterial strain XL1-Blue MRF' (Stratagene) to a density of ~115 plaque-forming colonies per cm^2, and then transferred onto nitrocellulose membrane (Osmonics, Minnetonka, MN). Membranes were then hybridized overnight at 65°C with the radioactive probe, washed according to the method of Sambrook and Russell (2001), and exposed to X-ray film for 48 h. Positive clones from this first screen were isolated and plated to a density of less than 1.5 plaque-forming colonies per cm^2, which allowed isolation of single, positive clones. After the third screening, five positive clones were isolated. The cDNA insert was excised from the Lambda Uni-ZAP XR vector using an ExAssist helper phage (Strategene). It was then recircularized to generate subclones in the pBlue-Script SK(+) phagemid vector. The phagemid DNA was then extracted using a QIAprep MiniPrep system (QIAGEN, Valencia, CA) and the cDNA sequence determined at the Sequencing and Genotyping Facility at Kansas State University, Manhattan, KS.

Northern Blot Analysis

Northern blot analysis was carried out using the NorthernMax kit (Ambion, Austin, TX) according to the manufacturer's instructions. Total RNA (30 mg/lane) and poly(A) RNA (3.0 µg/lane) were separated on a 1% agarose gel and transferred to a BrightStar-Plus (Ambion) nylon membrane. The membrane was then hybridized overnight at 42°C with the radioactive probe. After the blot was washed, it was exposed to Kodak X-Omat AR film for 6 h at –80°C with an intensifying screen.

Sequence Analysis

Similarities of the amino acid sequence deduced from the cytochrome c oxidase subunit Va cDNA were searched by using the internet server at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). SeqWeb version 2 GCG of the Sequence Analysis Package version 10 (Genetic Computer Group, Madison, WI) was used to compare similar sequences. The sequence was deposited in the GenBank database.

RESULTS AND DISCUSSION

Cytochrome c Oxidase Subunit Va cDNA Sequence

Using hemi-nested PCR and degenerate primers, 306-bp and 201-bp DNA fragments were generated from the first and second rounds of PCR, respectively (Fig. 1). The deduced amino acid sequence of the 201-bp fragment showed 61% identity with the corresponding region of the cytochrome c oxidase subunit Va from the fruit fly (*Drosophila melanogaster*). This fragment was used as a homologous probe to screen 3.8×10^{12} pfu/ml recombinant clones from an amplified *R. dominica* cDNA library. Five positive clones were isolated. Sequencing of these clones at the 5′ end resulted in identical nucleotide sequences. It is likely that these clones are the result of the cDNA library amplification. Complete Sequence of two of these clones confirmed their exact nucleotide sequence match.

The complete sequence of these clones consists of 693-bp including an open reading frame of 450-bp (Fig. 2). The start codon is flanked by the purine adenine at position –3 and a cytosine at position +4, which facilitates translation (Kozak, 1987). The termination codon TAA is located between bp 527–529 and is followed by a non-translated region of 109-bp including a 49-bp poly(A) tail. The untranslated region also contains the ATTTA sequence, which may be involved in the cytoplasmatic stability of the poly(A) RNA (Shaw and Kamen, 1986). The polyadenylation signal TATAAA was predicted by GENESCANW (Burger and Karlin, 1997) and is located 25-bp upstream from the poly(A) tail.

Northern blot analysis of the cytochrome c oxidase subunit Va transcript in *R. dominica* adults showed a single RNA species of about 4.9 kb in length (Fig. 3). Our results suggest the existence of a long 5′-untranslated region in the poly(A) RNA. In humans and mice, the transcript sizes of the subunit Va are 750-bp (Rizzuto et al., 1988) and 1.1 kb (Nielsen, et al., 1989), respectively. Apparently, these transcripts are much smaller than that in *R. dominica*. The transcript size of the cytochrome c oxisae subunit Va in *D. melanogaster* also appears to be much smaller than that in *R. dominica* since the genomic sequence for the same gene in the former species is only 950 bp (Adams et al., 2000). Because the annotation for the cytochrome c oxidase subunit Va in African malaria mosquito (*Anopheles gambiae*) is not completed (Holt et al., 2002), it is not possible to predict whether or not the transcript size of the cytochrome c oxidase subunit Va in *R. dominica* is larger than that in *A. gambiae*. Our study indicated, however, that the poly(A) RNA accounts for only approximately 0.01% of total RNA in lesser grain borer adults, since similar band intensities for the cytochrome c oxidase subunit Va transcript were observed in Northern blot analysis with either 3.0 μg of poly(A) RNA or 30 mg of total RNA (Fig. 3).

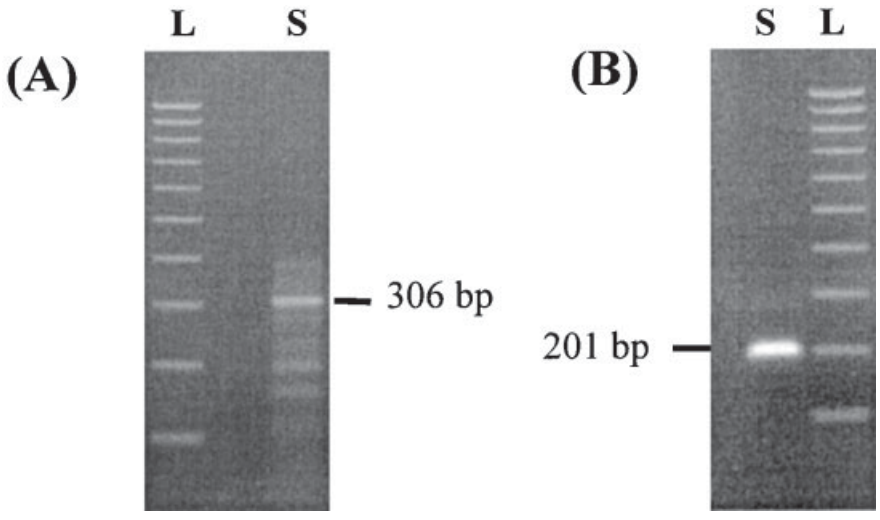


Fig. 1. Hemi-nested PCR using degenerate primers. **A:** First round of PCR. **B:** Second round of PCR. Ten microliters of the PCR reaction (S) from each round was run on 2% agarose gel. A 100-bp ladder (L) was used as a size marker.

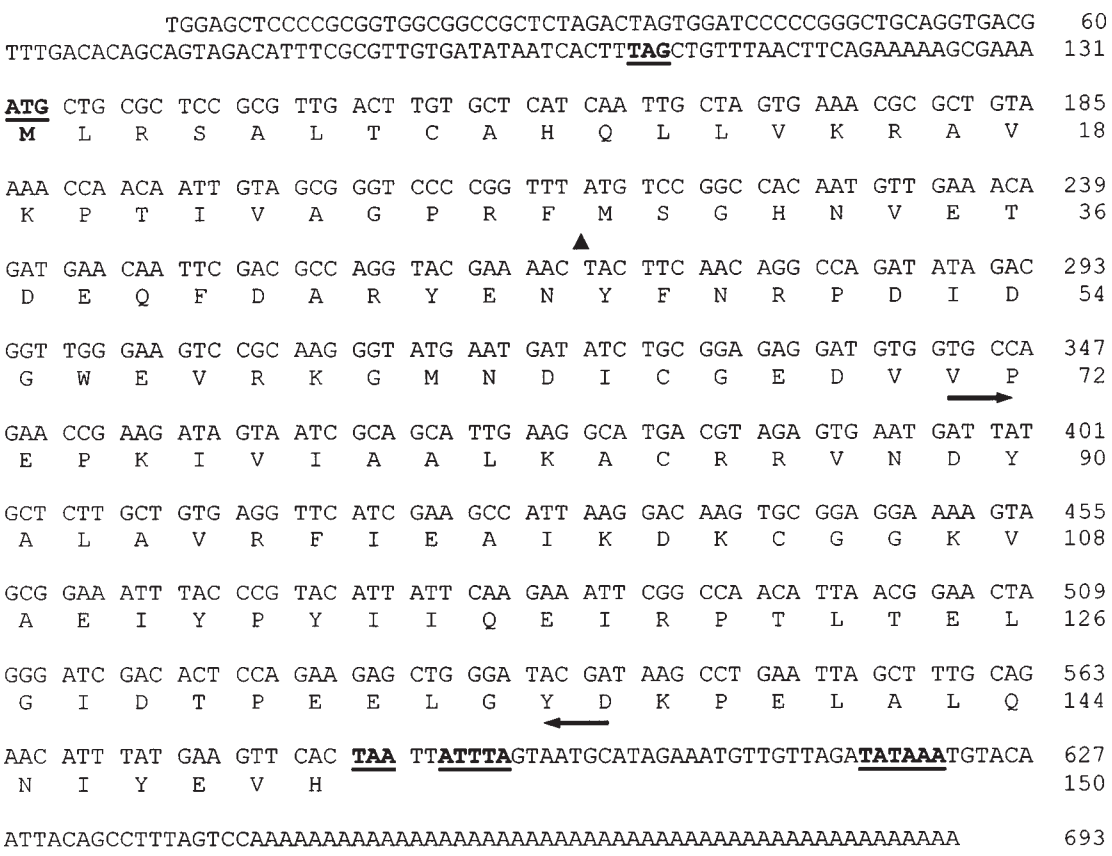


Fig. 2. Nucleotide and deduced amino acid sequences of the cytochrome c oxidase subunit Va cDNA isolated from *R. dominica*. The underlined sequences are TAG for stop codon upstream from the starting codon, ATG for start codon, TAA for stop codon at the end of the coding sequence, ATTTA for conserved sequence, and TATAAA for polyadenylation signal. Arrowhead: Predicted leading peptide cleavage site. The sequence between arrows represents the 201-bp probe. This sequence was deposited in the GenBank (accession number: AF420467).

Putative Cytochrome c Oxidase Subunit Va Protein

The open reading frame of the cloned cDNA encodes a protein of 150 amino acid residues (Fig. 2), which corresponds to the precursor protein for cytochrome c oxidase subunit Va. Analysis of the protein sequence by MitoProt II (Claros and Vincens, 1996) predicted a 28-residue leader sequence at the 5′ end and a mature protein of 122 amino acid residues (Fig. 2). The leader sequence in most nuclear subunits of the cytochrome c oxidase is necessary for the translocation of the protein into the inner membrane of the mitochondrion (Glaser et al., 1990). Upon translocation, the signal sequence is removed and the protein is folded into its functional form (Pfanner et al., 1994).

The cDNA-deduced precursor subunit Va of cytochrome c oxidase from *R. dominica* was aligned with those from other animal species deposited in GenBank using CLUSTALW (Thompson, et al., 1994) (Fig. 4). The alignment shows that 43% of amino acid residues are conserved among insects, including *A. gambiae* and *D. melanogaster*. Although 44% of amino acid residues are conserved between *R. dominica* and mammals, including rat (*Rattus norvegicus*), mouse (*Mus musculus*), and human (*Homo sapiens*), only 31% of all amino acid residues are conserved among the six animal species (Fig 4). Most of the conserved amino acids

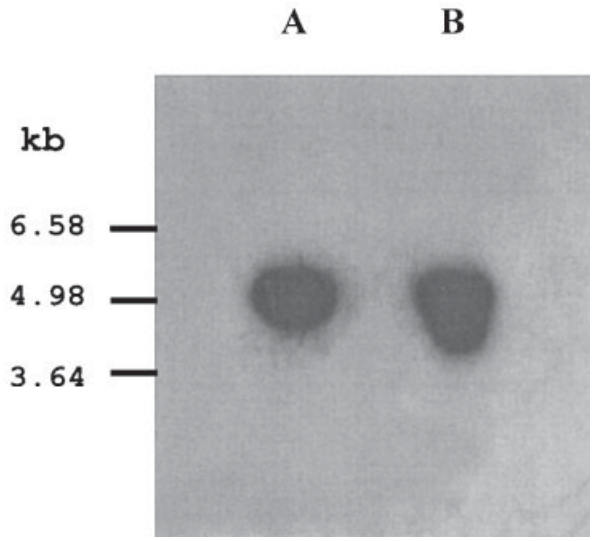


Fig. 3. Northern blot analysis of poly(A) RNA (3.0 μg, **lane A**) and total RNA (30 mg, **lane B**) from *R. dominica*. Poly(A) RNA and total RNA were separated on 1% agarose/formaldehyde gel, transferred to a BrightStart-Plus nylon membrane, and then hybridized with α-[^{32}P]dATP-labeled 201-bp cDNA probe.

are found in the second half of the sequence, toward the 3′ end, which encompasses the putative mature protein. Since the function of the nuclear subunits is unclear, the function of these conserved amino acids in cytochrome c oxidase subunit Va remains to be determined.

Mature Peptide Comparison and Evolutionary Relationship

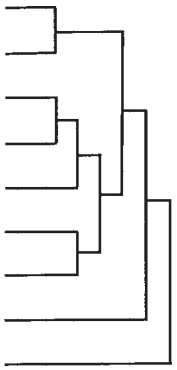
A search of non-redundant BLASTX on GenBank database was conducted to find homologous sequences to cytochrome c oxidase subunit Va from *R. dominica*. The most similar sequences in the database include those from the bovine (*B. taurus*) (Tsukihara et al., 1996), fruit fly (*D. melanogaster*) (Caggese et al., 1999), human (*H. sapiens*) (Rizzuto et al., 1988), mouse (*M. musculus*) (Nielsen et al., 1989), mosquito (*A. gambiae*) (Holt et al., 2002), nematode (*Caenorhabditis elegans*) (Anonymous, 1998), rat (*R. norvegicus*) (Droste et al., 1989), and yeast (*Saccharomyces cerevisiae*) (Cumsky et al., 1987) (Table 1). When the GCG Pileup program was used to compare the putative mature cytochrome c oxidase subunit Va from a variety of organisms to that of *R. dominica*, it showed that the subunit is most closely related to that from *A. gambiae*, with 80% similarity and 73% identity. Unexpectedly, *R. dominica* subunit sequence shows only 70% similarity and 59% identity to that of *D. melanogaster*, which are lower percentages than those for mammals (Table 1). Evolutionary distance analysis (GCG: Kimura protein distance) also indicated that cytochrome c oxidase subunit Va from *R. dominica* was most closely related to that of *A. gambiae* (Table 1).

```
R.d.  MLRSALTCAHQLLVK---------RAVKPTIVAG---PRFMSGHNVETDEQFDAR   43
A.g.  MLRFAAGRVLGGLRS---------AAGLKSSQPMVGAMVVRHSHSNETDEEFDKR   43
D.m.  MLSITARNLASALRS---------SLVGTSSR---VAAVRCLHGTEESAEEFDKR   43
H.s.  MLGAALRRCAVAATTRADPRGLLHSARTPGPAVAIQSVRCYSHGSQETDEEFDAR   55
M.m.  MLAAALRRCTAAAAR-----GLLHPASAPSPAAAVCSIRCYSHGSHETDEEFDAR   50
R.n.  MLAAALRRCTAAAAAR----GLLHPVSAPSPAAAVCSIRCYSHGSHETDEEFDAR   51
      **                                                * * ** *

R.d.  YENYFNRPDIDGWEVRKGMNDICGEDVVPEPKIVIAALKACRRVNDYALAVRFIE   98
A.g.  YEAYFNRPEIDGWEARKAMNDLLGMDLVPEPKIIVSALKACRRLNDYALAVRFLE   98
D.m.  YEKYFSREGIDGWEIRKGMNDLLGMDLVPSPKIIEAGLRASRRVNDIALAIRWLE   98
H.s.  WVTYFNKPDIDAWELRKGINTLVTYDMVPEPKIIDAALRACRRLNDFASLVRILE  110
M.m.  WVTYFNKPDIDAWELRKGMNTLVGYDLVPEPKIIDAALRACRRLNDFASAVRILE  105
R.n.  WVTYFNKPDIDAWELRKGMNTLVGYDLVPEPKIIDAALRACRRLNDFASAVRILE  106
         **    ** ** **  *     * ** ***    * * ** ** *   *  *

R.d.  AIKDKCGGKVAEIYPYIIQEIRPTLTELGIDTPEELGYDKPELALQNIYEVH     150
A.g.  GVKDKCGDKTNEIYPYLLQEIRPTLTELGIDTPEELGYDQPELALKSVYDMH     153
D.m.  GCKDKCGDQKATLYPYLLEKITPTLQELGIPTIEELGYDKPELALKSVYDA-     149
H.s.  VVKDKAG-PHKEIYPYVIQELRPTLNELGISTPEELGLDKV-----------     150
M.m.  VVKDKAG-PHKEIYPYVIQELRPTLNELGISTPEELGLDKV-----------     145
R.n.  VVKDKAG-PHKEIYPYVIQELRPTLNELGISTPEELGLDKV-----------     146
        *** *      ***      *** **** * **** *
```

Fig. 4. Predicted amino acid sequence of cytochrome c oxidase subunit Va from *R. dominica* (*R.d.*), and alignment with corresponding sequences from *A. gambiae* (*A.g.*), *D. melanogaster* (*D.m.*), *H. sapiens* (*H.s.*), *M. musculus* (*M.m.*), and *R. norvegicus* (*R.n.*). Identical amino acid residues among the six species are marked by an asterisk (*).

TABLE 1. Comparison of Similarity and Evolutionary Distance Relationship (GCG: Kimura Protein Distance) of Cytochrome c Oxidase Subunit Va From Nine Different Organisms

Organism	Similarity (%)	Identity (%)	Evolutionary distance	Reference
D. melanogaster	70	59		Caggese et al. (1999)
R. norvegicus	70	60		Droste et al. (1989)
B. taurus	77	67		Tsukihara et al. (1996)
M. musculus	77	67		Nielsen et al. (1989)
H. sapiens	74	64		Rizzuto et al. (1988)
A. gambiae	80	73		Holt et al. (2002)
R. dominica	—	—		
C. elegans	68	54		Anonymous (1998)
S. cerevisiae	19	11		Cumsky et al. (1987)



ACKNOWLEDGMENTS

The authors thank Jian-Rong Gao, Yu-Cheng Zhu, and Michele Zuercher for their technical assistance, and Nannan Liu and Michael E. Scharf for their review of an earlier draft of this article. This study was a cooperative investigation between USDA/ARS and Kansas State University and was supported by USDA/ARS. Mention of a proprietary product does not constitute a recommendation or endorsement by USDA. USDA is an equal opportunity/affirmative action employer, and all agency services are available without discrimination. This manuscript is Contribution No.03-278-J from the Kansas Agricultural Experiment Station. The lesser grain borer voucher specimens (No. 077) were deposited in the Museum of Entomological and Prairie Arthropod Research at Kansas State University, Manhattan, KS 66506.

LITERATURE CITED

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Ayala J, Dowdy AK, Beeman RW, Zhu KY. 2000. The genome sequence of *Drosophila melanogaster*. Science 287:2185–2195.

Anonymous 1998. Genome sequence of the nematode *C. elegans*: A platform for investigating biology. The *C. elegans* Sequencing Consortium. Science 282:2012–2018.

Arnold S, Goglia F, Kadenbach B. 1998. 3,5-Diiodothyronine binds to subunit Va of cytochrome-c oxidase and abolishes the allosteric inhibition of respiration by ATP. Eur J Biochem 252:325–330.

Burger C, Karlin S. 1997. Prediction of complete gene structure in human genomic DNA. J Mol Biol 268:78–94.

Caggese C, Ragone G, Perrini B, Moschetti R, De Pinto V, Caizzi R, Barsanti P. 1999. Identification of nuclear genes encoding mitochondrial proteins: isolation of a collection of *D. melanogaster* cDNAs homologous to sequences in the Human Gene Index database. Mol Gen Genet 261:64.

Capaldi RA. 1990. Structure and function of cytochrome c oxidase. Annu Rev Biochim Biophy Acta 1352:174–192.

Chaudhry MQ. 1997. A review of the mechanism involved in the action of phosphine as an insecticide and phosphine resistance in stored-product insects. Pestic Sci 49: 213–228.

Chefurka W, Kashi KP, Bond EJ. 1976. The effect of phosphine on electron transport in mitochondria. Pestic Biochem Physiol 6:65–84.

Claros MG, Vincens P. 1996. Computational method to predict mitochondrially imported proteins and their targeting sequence. Eur J Biochem 241:779–786.

Colman A, Robinson C. 1986. Protein import into organelles: hierarchical targeting signals. Cell 46:321–322.

Cumsky MG, Trueblood CE, Ko C, Poyton RO. 1987. Structural analysis of two genes encoding divergent forms of yeast cytochrome c oxidase subunit V. Mol Cell Biol 7:3511–3519.

Droste M, Schon E, Kadenbach B. 1989. Nucleotide sequence of cDNA encoding subunit Va from rat heart cytochrome c oxidase. Nucleic Acids Res 17:4375.

Glaser SM, Miller BR, Cumsky MG. 1990. Removal of hydrophobic domain within the mature portion of mitochondrial inner membrane protein causes its mislocalization to the matrix. Mol Cell Biol 10:1873–1881.

Grossman LI, Lomax MMI. 1997. Nuclear gene for cytochrome c oxidase. Biochim Biophy Acta 1352:174–192.

Hatefi Y. 1985. The mitochondrial electron transport and oxidative phosphorilation system. Ann Rev Biochem 54:1015–1069.

Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R, Nusskern DR, et al. 2002. The genome sequence of the malaria mosquito *Anopheles gambiae*. Science 298:129–149.

Hurt EC, van Loon APGM. 1986. How proteins find mitochondria and intramitochondrial compartments. Trends Biochem Sci 18:39–54.

Iwata S, Ostermeir C, Ludwig B, Michel H. 1995. Structure at 2.4 Å resolution of cytochrome c oxidase from *Paracoccus denitrificans*. Nature 376:660–669.

Kadenbach B. 1986. Regulation of respiration and ATP synthesis in higher organisms: hypothesis. J Bionerg Biomembr 18 39–54.

Kadenbach B, Reimann A. 1992. Cytochrome c oxidase: tissue-specific expression of isoforms and regulation of activity. In: Ernster L, editor. Molecular mechanisms in bioenergetics: new comprehensive biochemistry. New York: Elsevier Science Publishers. p 241–263.

Kozak M. 1987. An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res 15: 8125–8132.

Myers D, Palmer G. 1988. The kinetic mechanism(s) of cytochrome oxidase: technique for their analysis and criteria for their evaluation. Ann NY Acad Sci 550:85–97.

Nakakita H. 1987. The mode of action of phosphine. J Pestic Sci 12:299–309.

Nakakita H, Katsumata Y, Ozawa T. 1971. The effect of phosphine on respiration of rat liver mitochondria. J Biochem 69:589–594.

Nielsen PJ, Ayane M, Kohler G. 1989. Nucleotide sequence of cDNA encoding mouse cytochrome c oxidase subunit Va. Nucleic Acids Res 17:6723.

Pfanner N, Craig EA, Meijer M. 1994. The protein import machinery of the mitochondrial inner membrane. Trends Biochem Sci 19:368–372.

Rizzuto R, Nakase H, Zeviani M, DiMauro S, Schon EA. 1988. Subunit Va of human and bovine cytochrome c oxidase is highly conserved. Gene 69:245–256.

Sambrook J, Russell DW. 2001. Molecular cloning: A laboratory manual, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Shaw G, Kamen R. 1986. A conserved adenine uridine sequence from the 3′ untranslated region of granulocyte-monocyte colony stimulating factor messenger RNA mediates selective messenger RNA degradation. Cell 46:659–668.

Soboll S. 1993. Thyroid hormones action on mitochondrial energy transfer. Biochim Biophys Acta 1144:1–16.

Taanman J-W, Williams SL. 2001. Assembly of cytochrome c oxidase: What can we learn from patients with cytochrome c oxidase deficiency? Biochem Soc Trans 29:446–451.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680.

Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa S. 1996. The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 Å. Science 227:1136–1144.

Zhang Y-Z, Capaldi RA. 1988. Subunit composition of the transmembrane parts of beef heart cytochrome c oxidase. Ann NY Acd Sci 550:1129–1138.

Zhu KY, Clark JM. 1995. Cloning and sequencing of a cDNA encoding acetylcholinesterase in Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Insect Biochem Mol Biol 25: 1129–1138.